Multiplicity of 3-Ketosteroid-9 alpha-Hydroxylase Enzymes in Rhodococcus rhodochrous DSM43269 for Specific Degradation of Different Classes of Steroids

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Supplemental Table 1. Plasmids used in this study.

Construct	Description	Reference/origin
pRRE1	*E. coli-Rhodococcus* shuttle vector	Rosłoniec *et al*. (2009) (26)
pKSH602	Clone isolated from genomic library of DSM43269 carrying *kshA1*	Wilbrink *et al*., submitted
pA1rho9	pRRE1 carrying DSM43269 genomic region of *kshA1* for functional complementation of RG32; 6,296 bp *Eco*RI fragment of pKSH602 ligated in pRREI *Eco*RI	This study
pKSH509	Clone isolated from genomic library of DSM43269 carrying *kshA2*	Wilbrink *et al*., submitted
pA2rho5	pRRE1 carrying DSM43269 genomic region of *kshA2* for functional complementation of RG32; 5,291 bp *Eco*RV*/Hind*III fragment of pKSH509 ligated in pRREI *Eco*RV*/Hind*III	This study
pKSH800	Clone isolated from genomic library of DSM43269 carrying *kshA3*	Wilbrink *et al*., submitted
pZero2.1	General cloning vector	Invitrogen
pKSH801	DSM43269 genomic region of *kshA3;* ± 3 kb BamHI fragment of pKSH800 ligated in pZero *Bam*HI	This study
pA3rho5	pRRE1 derivative carrying DSM43269 genomic region of *kshA3* for functional complementation of RG32; 2,401 bp *Eco*RV fragment of pKSH801 ligated in pRREI *Eco*RV	This study
pKSH628	Clone from genomic library of DSM43269 carrying *kshA4*	Wilbrink *et al*., submitted
pKSH629	Clone from genomic library of DSM43269 carrying *kshA4*	Wilbrink *et al*., submitted
pA4rho3	DSM43269 genomic region of *kshA4*; ligation of 3,182 bp *Xmn*I*/Dra*III fragment of pKSH628 with 4,320 bp *Xmn*I*/Dra*III fragment of pKSH629	This study
pA4rho15	pRRE1 carrying DSM43269 genomic region of *kshA4* for functional complementation RG32; 4,251 bp *EcoRI/HindIII* fragment of pA4rho3 ligated in pRRE1 *EcoRI/HindIII*	This study
pBrho5	*kshB* in pET15b	Petrusma *et al*. (2009) (24)
pBSKS	pBluesript (II), cloning vector	Stratagene
pKSH814	cloning vector carrying *kshA* ; KshARho-1-F + -R PCR (Vent polymerase) product (*kshA1*) ligated in pBSKS *Eco*RV	This study
pET15b	T7 expression plasmid	Novagen
pKSH816	*kshA1* expression plasmid; 1,190 bp *Nde*I*/Hind*III fragment (*kshA1*) pKSH 814 ligated in pET15b *Nde*I*/Hind*III.	This study
pA1rho3	co-expression construct *kshA1-kshB;* 1,289 bp *Hind*III*/Xba*I fragment pKsh816 ligated in pBrho5 *Hind*III*/Spe*I	This study
pKSH660	cloning vector carrying *kshA* ; KshARho-2-F2 + -R PCR (High Fidelity polymerase) product (*kshA2*) ligated in pZero *Eco*RV	This study
pKSH661	*kshA2* expression plasmid; 1,268 bp *Nde*I*/Hind*III fragment of pKSH 660 ligated in pET15b *Nde*I*/Hind*III	This study
pA2rho2	co-expression construct *kshA2-kshB;* 1,216 bp *Hind*III*/Xba*I fragment of pBrho5 ligated in pKsh661 *Hind*III*/Spe*I	This study
pCR-XL-TOPO	cloning vector	Invitrogen
pKSH827	cloning vector carrying *kshA3*; KshARho-3-F + -R PCR (Expand polymerase) product (*kshA3*) ligated in pCR-XL-TOPO	This study
pKSH829	*kshA3* expression plasmid; 1,216 kb *Nde*I*/Bam*HI fragment of pKSH 827 in pET15b *Nde*I*/Bam*HI	This study
pA3rho2	co-expression construct *kshA3-kshB;* 1,500 bp *Hind*III*/Xba*I fragment of pBrho5 in pKsh829 *Hind*III*/Spe*I	This study
pA4rho4	KshA4 and KshB co-expression vector	Petrusma *et al*. (2009) (24)
pKSH920	cloning vector carrying *kshA5*; *kshA5* PCR product obtained using primers KshARho-5-F and KshRho-5-R ligated in pZero *Eco*RV	This study
pKSH921	*kshA5* expression plasmid; 1,227 bp *Nde*I*/Bam*HI *fragment* of pKSH 920 ligated in pET15b *Nde*I*/Bam*HI	This study
pA5rho2	co-expression construct *kshA5-kshB;* 1,216 bp *Hind*III*/Xba*I fragment of pBrho5 ligated in pKsh921 *Hind*III*/Spe*I	This study